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The Oxidation of Myoglobin to Metmyoglobin by Oxygen

3. KINETIC STUDIES IN THE PRESENCE OF CARBON MONOXIDE, AND AT DIFFERENT HYDROGEN-ION CONCENTRATIONS WITH CONSIDERATIONS REGARDING THE STABILITY OF OXYMYOGLOBIN

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In Parts 1 and 2 (George & Stratmann, 1952*a*, *b*) it was shown that the oxidation of myoglobin to metmyoglobin by oxygen resembles very closely the corresponding oxidation of haemoglobin (Brooks, 1931, 1935). Only quantitative differences were found: in other respects the systems were identical. At constant oxygen pressure the oxidation rate is first order with respect to unoxidized myoglobin or haemoglobin; the rate constant at low oxygen pressures is proportional to the product of the concentrations of reduced myoglobin (Mb) and oxymyoglobin (MbO_2), or of reduced haemoglobin (Hb) and oxyhaemoglobin (HbO_2); at high oxygen pressures the rate constant is proportional either to $[MbO_2]$ or $[HbO_2]$, or to $[MbO_2]^2$ or $[HbO_2]^2$. Because results could not be obtained with sufficient precision at high oxygen pressures to decide between these alternatives, an attempt has now been made to decide between them by making use of the inhibition of myoglobin oxidation by carbon monoxide.

Brooks (1931) found that haemoglobin oxidation in air occurs more rapidly in more acid solutions. Similar experiments with myoglobin have now been carried out at both high and low oxygen pressures, over a range of hydrogen-ion concentrations.

Finally, some of the factors which contribute to the stability of oxymyoglobin and oxyhaemoglobin with respect to oxidation are discussed.

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MATERIALS AND METHODS

The myoglobin used was prepared as described by George & Stratmann (1952*a*). In the inhibition experiments, the myoglobin solutions were heavily buffered with K_2HPO_4 and KH_2PO_4, ratio 0·159/0·841, giving a total concentration of phosphate ions of 0·6 M with a pH of 5·69 at 30°. In the experiments in which pH was varied, the myoglobin was buffered with phosphate buffer mixtures prepared from NaH_2PO_4 and NaOH, giving pH values over the range 5·35–6·64. The ionic strengths of these buffer solutions were brought to 0·8, the same value as that of the 0·6 M potassium phosphate buffer, by the addition of appropriate quantities of NaCl. The pH of the myoglobin solutions was determined before each kinetic experiment using a glass electrode, calibrated with a 0·05 M potassium hydrogen phthalate buffer.

O_2, N_2, and CO were obtained from cylinders.

Kinetic measurements

The concentration of myoglobin was $1·68 \times 10^{-4}$ M. The reaction vessel, flow apparatus, and the procedure for analysis throughout the course of an experiment were as described by George & Stratmann (1952*a*, *b*). The results were recorded as the percentage of unoxidized myoglobin at various times from the start of the reaction. The log. of the percentage of unoxidized myoglobin was plotted against time in hours and a first-order rate constant, $k_{obs.}$ ($hr.^{-1}$), calculated from the slope of the line. In the experiments in which pH was varied, series of values for $k_{obs.}$ were obtained at two partial pressures of oxygen, 4 and 760 mm. In the inhibition experiments, CO–O_2 mixtures were used with partial pressures of CO(p_{CO}) between 0 and 45 mm. and of O_2(p_{O_2}) between 760 and 715 mm. All experiments were carried out at 30°.

RESULTS

Carbon monoxide inhibition

The oxidation of Mb to MetMb in mixtures of O_2 and CO of total pressure 760 mm., in which p_{CO} was varied from 10 to 45 mm. was found to be first order with respect to unoxidized myoglobin as is the case in the absence of carbon monoxide. This is illustrated in Fig. 1 where log. percentage (MbO_2 + MbCO) is plotted against time for experiments with p_{CO} 10 and 25 mm. Fig. 2 shows the relationships between the observed velocity constant $k_{obs.}$ (hr.$^{-1}$) and p_{CO}. The experiments at the highest values of p_{CO}, 35 and 45 mm., where half-reaction times were of the order of 7 hr., were less reproducible than the others, and the curve in Fig. 2 has been dotted in this region to indicate this uncertainty. The value of p'_{CO}, the partial pressure of carbon monoxide required to halve the uninhibited rate constant was 21 ± 2 mm.

These results can now be analysed to see what light they throw on the problem of which of the two alternative rate equations for the oxidation at high p_{O_2}, previously found to fit the experimental results equally well, is the correct one (George & Stratmann, 1952*b*),

$$-d[Fe_p^{2+}]/dt = 0{\cdot}30(1-\alpha)^2\,[Fe_p^{2+}], \quad (1)$$

or

$$-d[Fe_p^{2+}]/dt = 0{\cdot}30(1-\alpha)\,[Fe_p^{2+}]. \quad (2)$$

In these equations $[Fe_p^{2+}]$ stands for the total concentration of unoxidized myoglobin, i.e. [Mb] + $[MbO_2]$, and $(1-\alpha)$ gives the fraction present as MbO_2.

For the present experimental conditions, where the values of p_{O_2} and p_{CO} are such that all the myoglobin can be assumed to be present as MbO_2 and MbCO, it follows that

$$(1-\alpha) = 1\Big/\left\{\frac{Mp_{CO}}{p_{O_2}} + 1\right\},$$

where M, the partition constant given by

$$[MbCO]\,p_{O_2}/[MbO_2]\,p_{CO},$$

is left uncorrected for the different solubilities of O_2 and CO. Hence, according to equations 1 and 2 the rate constant is halved when either $(1-\alpha)^2$ or $(1-\alpha) = \frac{1}{2}$, corresponding to $Mp'_{CO}/p_{O_2} = 0{\cdot}41$ or 1. Using p'_{CO} 21 ± 2 mm. as determined above, and $p_{O_2} = 740$ mm. gives $M = 14{\cdot}5 \pm 1{\cdot}5$ or $M = 35 \pm 4$ respectively.

An attempt was made to determine M under identical experimental conditions by spectrophotometric estimation of MbO_2, MbCO and MetMb, in samples of myoglobin equilibrated in O_2–CO mixtures. Optical density measurements were made at 960 and 660 mμ. in the near infrared, where there are greater relative differences in absorption spectra than in the visible or Soret regions, and M was calculated by the method used by George & Stratmann (1952*b*) to obtain the oxygenation equilibrium constant. Very poor reproducibility was obtained, the mean of nine determinations giving 28 ± 8. It is in part attributable to the very large contribution to optical density from MetMb, unavoidably formed during equilibration, whose molar extinction coefficient at 960 mμ. was found to be 850, compared with 288 for MbO_2 and 5·3 for MbCO. With this method, any error in correcting for MetMb appears as a large error in M. It was therefore decided to rely on values in the literature, although these were obtained under different experimental conditions.

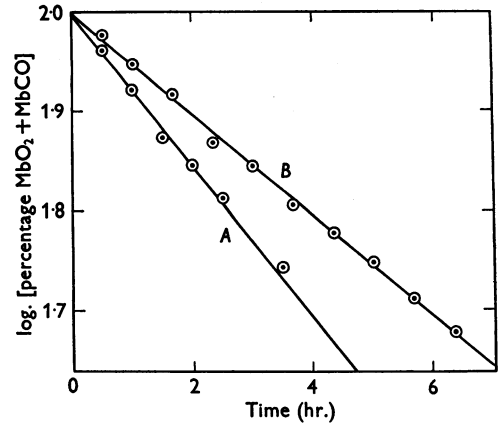

Fig. 1. First-order plots for the oxidation of myoglobin to metmyoglobin by oxygen at 30°, in 0·6 M phosphate buffer pH 5·7 in the presence of carbon monoxide. *A*, 10 mm. CO, 750 mm. O_2; *B*, 25 mm. CO, 735 mm. O_2.

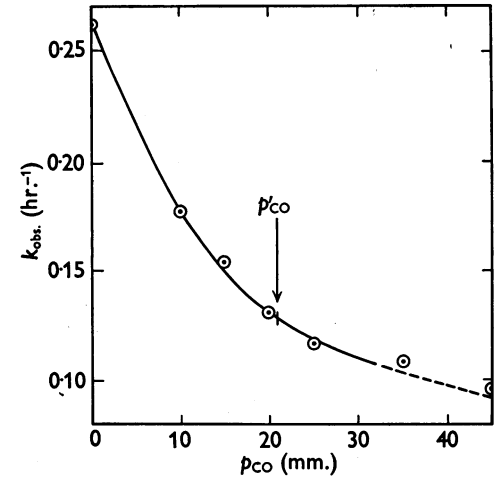

Fig. 2. The variation of the first-order rate constant, $k_{obs.}$ with the partial pressure of carbon monoxide, p_{CO}. The values for 35 and 45 mm. are less reliable than the others. The arrow indicates p'_{CO}, the partial pressure required to give a rate constant one half of the uninhibited rate constant.

Theorell (1934*a*) obtained values for M at pH 6·95 of 19·3 ± 3·0 at 20° and 13·8 ± 2·8 at 37°, from which the value 15·8 at 30° can be calculated using the van't Hoff isochore. This value has an error of about 18 %, i.e. ± 2·8. It is unlikely that M would have an appreciably different value at the pH of 5·7 used in the present experiments as the following considerations show. First, the similarities between myoglobin and haemoglobin reactions extend to the way M varies with temperature; with haemoglobin the ratio of the values at 15 and 37° is 1·60 (Anson, Barcroft, Mirsky & Oinuma, 1925) compared with the value of 1·55 for myoglobin calculated from Theorell's data given above. Secondly, M for haemoglobin is pH independent, for although the equilibrium constants for HbO_2 and HbCO formation both show quite a marked pH dependence, it is of the same kind in both cases (Wyman, 1948). Some confirmation that the value of M is between 10 and 20 for myoglobin at 20° comes from Millikan's (1936) determinations of the velocity constants for the formation and dissociation of MbO_2 and MbCO at pH 7·4, namely $1·9 \times 10^7$ and 3×10^5 M^{-1} sec.$^{-1}$, and 37 and 0·04 sec.$^{-1}$ respectively. These constants are subject to an average error of 28 %, but as they stand they give $M = 14·6$. Using the temperature coefficient obtained from Theorell's data this becomes 12·0 at 30°.

There seems to be little doubt that under the present experimental conditions M would have a value somewhere between 13 and 19, and so equation 1, which gives $M = 14·5 \pm 1·5$, is to be preferred to equation 2, which gives $M = 35 \pm 4$, for the purpose of expressing the oxidation kinetics at high oxygen pressures.

Hydrogen-ion dependence

The first-order velocity constants, k_{760} and k_4, for the oxidation of myoglobin to metmyoglobin by oxygen in phosphate buffer solutions of pH 5·35 to 6·64, with ionic strength 0·80, $p_{O_2} = 760$ and 74 mm., are plotted in Fig. 3 against the hydrogen-ion concentration.

As shown above, equation 1 in which $k_{obs.} = 0·30$ $(1-\alpha)^2$ hr.$^{-1}$, is to be preferred to equation 2 at high values of p_{O_2}. The corresponding equation at low values of p_{O_2} is $k_{obs.} = 2·62\alpha\ (1-\alpha)$ hr.$^{-1}$ (George & Stratmann, 1952*b*). Hence in analysing the variation of k_{760} and k_4 with $[H^+]$ it is first necessary to make allowance for the variation of $(1-\alpha)^2$ and $\alpha\ (1-\alpha)$ with $[H^+]$, since 760 and 4 mm. respectively fall in the ranges of p_{O_2} values where $k_{obs.}$ is given by these two expressions.

$(1-\alpha)$ and hence $(1-\alpha)^2$ is effectively constant at different pH values with $p_{O_2} = 760$ mm., since it is given by $p_{O_2}/(K_{O_2} + p_{O_2})$, where K_{O_2} is the dissociation constant of MbO_2, K_{O_2} having values of about 1 mm. for the present experimental conditions (Theorell, 1934*b*). Hence the variation of k_{760} with $[H^+]$ shown in Fig. 3 must arise through an ionization, or direct participation of hydrogen ions, at some stage in the actual oxidation mechanism.

Unlike $(1-\alpha)$, α is sensitive to changes in $[H^+]$ at all but extremely low values of $p_{O_2} < 0·05$ mm., because it is given by $K_{O_2}/(K_{O_2} + p_{O_2})$, and K_{O_2} shows a small but definite decrease as pH increases (Theorell, 1934*b*). log $(1/K_{O_2})$ plotted against pH is approximately linear, increasing by 0·098 and 0·116 per pH unit at 37 and 27° respectively. The difference is small, so rather than try to calculate a value for 30° based on ionization equilibria a mean value of 0·107 per pH unit has been taken, and from the value of $K_{O_2} = 1·13$ mm. at 30° and pH 5·7 obtained by George & Stratmann (1952*b*), the corresponding values of K_{O_2} have been calculated for each pH used in the present experiments. The true kinetic dependence on hydrogen-ion concentration of the velocity constants that go to make up k_4 can now be obtained by dividing k_4 by $K_{O_2} p_{O_2}/(K_{O_2} + p_{O_2})^2$, putting $p_{O_2} = 4$ mm. This new velocity constant will be called k_4'. The results of the calculations are summarized in Table 1, and the dotted line in Fig. 3 gives the plot of k_4' against $[H^+]$.

The plots in Fig. 3 show that with $p_{O_2} = 4$ and 760 mm. the variation of the rate constant is substantially less than the first-power variation, i.e. tenfold increase in K for a tenfold increase in $[H^+]$, which might result if the hydrogen ion were participating directly as a reactant at some stage in the oxidation process.

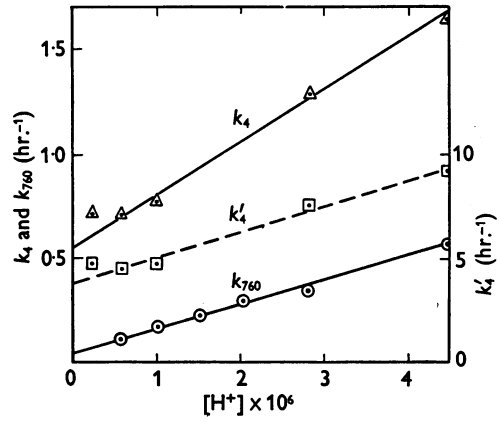

Fig. 3. The first-order velocity constants, k_4 and k_{760}, for the oxidation of myoglobin to metmyoglobin by oxygen in phosphate buffer solutions of pH 5·35 to 6·44 with ionic strength 0·8, at partial pressures of oxygen 4 and 760 mm. respectively, plotted against the hydrogen-ion concentration. The dotted line labelled k_4' is derived from the k_4 data by applying a correction described in the text.

Table 1. pH *and myoglobin oxidation*

Data for evaluating the hydrogen-ion dependence of the true oxidation velocity constant at low oxygen pressures, i.e k_4', using values of K_{O_2} calculated from a value of 1·13 mm. at pH 5·69 determined by George & Stratmann (1952*b*), and the variation of K_{O_2} with pH based on Theorell's measurements (1934*b*).

pH	k_4 (hr.$^{-1}$)	K_{O_2} (calc.) (mm.)	$\frac{4K_{O_2}}{(K_{O_2}+4)^2}$	$\frac{k_4(K_{O_2}+4)^2}{4K_{O_2}}=k_4'$
5·35	1·65	1·22	0·180	9·2
5·55	1·29	1·16	0·174	7·4
6·00	0·77	1·04	0·165	4·7
6·24	0·71	0·98	0·159	4·5
6·64	0·71	0·89	0·149	4·8

On the other hand, velocity constants in haemoprotein reactions often vary with $[H^+]$ because the ionization of one or more groups near the haem (or haemin) affect its reactivity. If K represents the ionization constant of one of these groups then its ionization can lead to a hydrogen-ion dependence of the form $[H^+]/(K+[H^+])$, $K/(K+[H^+])$, or some linear combination of the two, all of which can give a less than first-power variation with $[H^+]$. In haemoglobin compounds the pK values of such haem-linked ionizations are well established (Wyman & Ingalls, 1941), and recent calculations on data for myoglobin show it to have a linked group with a pK of about 6·6 in Mb and 6·3 in MbO_2 at temperatures of about 30° (George & Hanania, 1954). The data in Fig. 3 are not extensive enough to merit detailed mathematical analysis, but from the trend of the line it can be inferred that the variation of k_{760} with $[H^+]$ would follow if MbO_2 with the linked group in its conjugate acid form reacted several times more rapidly than the corresponding conjugate base. The variation of k_4' could be accounted for in a similar way, except that in this case both conjugate acid and conjugate base of Mb would have to be considered about equally reactive.

Brooks (1931) found that the velocity constant for haemoglobin oxidation in air also showed a less than first-order dependence on $[H^+]$, and it is very likely that this too could be explained as a consequence of haem-linked ionizations.

DISCUSSION

The rate equations previously proposed for the oxidation at low and high oxygen pressures are respectively:

$$\frac{-\mathrm{d}[Fe_p^{2+}]}{\mathrm{d}t}=\frac{k_a(1-\alpha)\,[Fe_p^{2+}]\,k_b\,\alpha[Fe_p^{2+}]}{k_c[Fe_p^{2+}]}, \qquad (3)$$

$$\frac{-\mathrm{d}[Fe_p^{2+}]}{\mathrm{d}t}=\frac{k_a(1-\alpha)\,[Fe_p^{2+}]\,k_b'\,(1-\alpha)\,[Fe_p^{2+}]}{k_c[Fe_p^{2+}]}, \qquad (4)$$

k_a is the velocity constant of a reaction of MbO_2 which initiates the oxidation, and k_b and k_b' are velocity constants of participating reactions of Mb and MbO_2 at low and high oxygen pressures respectively, all three of which are understood to be reactions of the haem iron atom. k_c is the velocity constant of another reaction in the overall process which apparently involves some other group in the molecule, because Mb and MbO_2 appear to undergo this reaction at equal rates. The inclusion of this reaction was essential to resolve the paradox that the rate at low pressures shows an oxygen pressure variation characteristic of the product

$$[Mb]\times[MbO_2], \text{ i.e. } \alpha(1-\alpha)\,[Fe_p^{2+}]^2,$$

whilst it is actually first order in $[Fe_p^{2+}]$.

In view of the first-order dependence on $[Fe_p^{2+}]$ at all oxygen pressures it is not surprising that the inhibited rate also shows the same behaviour (Fig. 1). The comparison of the partition constant M, obtained from the p_{CO} value necessary to halve the uninhibited rate constant, with the values obtained in gasometric experiments by Theorell (1934*a*) and from the velocity constants for O_2 and CO combination and dissociation (Millikan, 1936), supports a rate equation containing $(1-\alpha)^2$, as in equations 1 and 4, and not one containing $(1-\alpha)$, as in equation 2, to express the oxidation kinetics at high p_{O_2}. The first-order dependence on $[Fe_p^{2+}]$, and this quantitative check, support the general interpretation of the rate equations outlined above, for they imply that only the concentrations of the reactant species appearing in the numerator of the rate equation are diminished when carbon monoxide is present, and not that of the species in the denominator. In other words, the reaction with velocity constant k_c is unaffected when the haem iron atom combines with carbon monoxide just as it is apparently unaffected by the combination with oxygen.

The stability of oxymyoglobin with respect to its oxidation to metmyoglobin. Whatever the detailed mechanism of the oxidation, the fundamental electron transfer reaction (5)* will be among the

* In this chemical equation and those that follow, the symbol Fe_p^{2+} stands for reduced, i.e. uncombined, myoglobin, and not the unoxidized myoglobin as was the case in the previous kinetic equations 1 to 4.

most important governing the stability of oxymyoglobin,

$$Fe_p^{2+} + O_2 \rightarrow Fe_p^{3+} + O_2^-, \qquad (5)$$

for if this reaction is fast a rapid production of metmyoglobin will necessarily occur if it is not accompanied by significant back reactions regenerating myoglobin. Some indication of the speed can be inferred from an estimate of the heat of the reaction.

From a simple thermochemical cycle this can be shown to be $(E_{O_2} + S_{O_2^-}) - S_{O_2} - I_{Mb}$, where E_{O_2} is the electron affinity of the O_2 molecule, S_{O_2} and $S_{O_2^-}$ the solvation energies of the O_2 molecule and the O_2^- ion respectively, and I_{Mb} is the ionization potential of myoglobin in aqueous solution. Reasonable values of $(E_{O_2} + S_{O_2^-})$ and S_{O_2} are 79 and 3 kcal./g.mol. respectively and according to a very simple model, where only the change in charge on oxidizing Fe_b^{2+} to Fe_p^{3+} is considered, I_{Mb} would be about 90 kcal./g.mol. (George, 1952). Experimentally, I_{Mb} can be obtained from the heat of the cell reaction $Fe_p^{3+} + \frac{1}{2}H_2 \rightarrow Fe_p^{2+} + H^+$ which George & Hanania (unpublished work) calculated from preliminary results to be 89 ± 2 kcal./g.mol.; but an improved experimental technique now gives a higher value of 96 ± 2 kcal./g.mol. This must still be regarded as a provisional value because of experimental difficulties, but fortunately the uncertainty in no way affects the following argument. For if I_{Mb} is as high as 96 or as low as 89 kcal./g.mol., reaction (5) would be endothermic to the extent of 20 or 13 kcal./g.mol. respectively. Even if the reaction could occur with no additional activation energy, an abnormally high value of the temperature-independent factor A, in the Arrhenius equation $k = Ae^{-E/RT}$, of the order 10^{17} to 10^{21} l.mole^{-1} sec.$^{-1}$, compared with the usual value of about 10^{11} l.mole^{-1} sec.$^{-1}$, would be required to give k with a magnitude of 10^6 l.mole^{-1} sec.$^{-1}$ associated with 'fast' haemoprotein reactions.

The above calculations refer to the bimolecular reaction between myoglobin and oxygen resulting in electron transfer. For the fission of the complex itself with electron transfer as in reaction 6,

$$Fe_p^{2+}O_2 \rightarrow Fe_p^{3+} + O_2^- \qquad (6)$$

an additional factor, the exothermic formation of oxymyoglobin, must be considered. This enhances the resistance of myoglobin to oxidation by increasing the endothermicity of the overall electron transfer process as the following data show. The heat of formation of oxymyoglobin at the pH of 5·7 used by George & Stratmann (1952*b*) can be estimated as 19·5 kcal./g.mol. from Theorell's data (1934*b*). This becomes 16·4 kcal./g.mol. when corrected by 3·1 kcal./g.mol. for the heat of solution of oxygen, or 16 kcal./g.mol. to two significant figures. It therefore follows, by adding the heat of this reaction to that of 20 kcal./g.mol. calculated for reaction (5), that reaction (6) is endothermic to about 36 kcal./g.mol.

The detailed mechanism of these reactions may be envisaged with the help of the schematic potential energy diagram shown in Fig. 4. This diagram has been constructed from the thermochemical data and may be compared with that drawn up by Evans & Uri (1949) for their discussion of the formation of the $FeOH_{aq}^{2+}$ complex ion, from Fe_{aq}^{3+} and OH_{aq}^- ions, and the related reaction of Fe_{aq}^{2+} with the OH radical. The positions of state **A**, $Fe_p^{2+} + O_2$, and state **B**, $Fe_p^{2+}O_2$, are defined by the heat of formation of oxymyoglobin, and that of state **D**, $Fe_p^{3+} + O_2^-$, by the thermochemical cycle for reaction (5) above. State **C**, $Fe_p^{3+}O_2^-$, is placed 20 kcal./g.mol. above state **B** on the basis of the following consideration of the kinetic results given in George & Stratmann (1952*b*). The different form of the rate equations at low and high oxygen pressures, 4 and 760 mm., points to a common reaction in which Mb and MbO_2 respectively predominate, and in which oxidation of the iron occurs. The observed activation energies are 19 and 25 kcal./g.mol. respectively. The variation with temperature of the term involving K_{O_2} contributes an apparent activation energy of 14 kcal./g.mol. to that of the

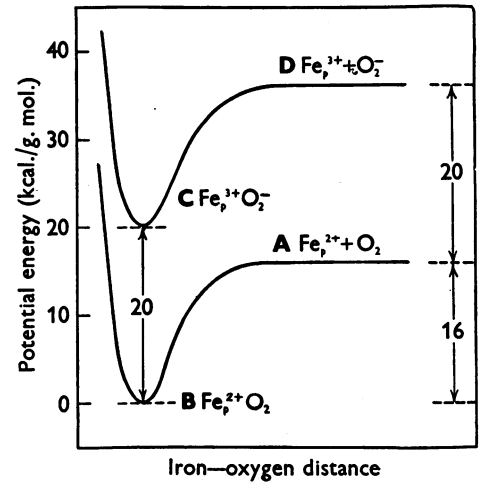

Fig. 4. Schematic potential energy diagram for the reaction of oxygen with myoglobin (Fe_p^{2+}) giving O_2^- and metmyoglobin (Fe_p^{3+}). In states **A** and **D**, Fe_p^{2+} and O_2, Fe_p^{3+} and O_2^- are infinitely separated. In state **B**, Fe_p^{2+} and O_2 have the internuclear separation characteristic of the stable oxymyoglobin molecule, $Fe^{2+}O_2$: state **C** is a hypothetical excited state, $Fe^{3+}O_2^-$, where electron transfer has occurred at this internuclear separation. States **A** and **B**, **C** and **D** are connected by lines drawn arbitrarily to give curves resembling the potential energy curves for diatomic molecules.

Mb reaction, so its real activation energy is 20 kcal./g.mol. less than that of the MbO_2 reaction. This difference of 20 kcal./g.mol. may be tentatively identified with the energy required to bring about the electron transfer $Fe_p^{2+}O_2 \rightarrow Fe_p^{3+}O_2^-$, since MbO_2 in this excited state could be expected to react as rapidly as Mb itself, where only electron transfer is required and no breaking of a Fe_p–O_2 covalent bond, in view of the high reactivity of the O_2^- in electron-transfer reactions.

Reaction 5 thus involves a change from state **A** to **B**, **B** to **C**, and finally from **C** to **D**, whereas reaction 6 only requires the change from **B** to **C**, and **C** to **D**. It is clear from Fig. 4, that because of the different initial state, reaction 5 is favoured to the extent of some 16 kcal./g.mol., provided that the exothermic heat of formation of $Fe_p^{2+}O_2$, the 16 kcal./g.mol., is not dissipated before electron transfer to state **C** has occurred. Summing up, in contrast to other classes of haemoproteins and haem derivatives, the exothermic formation of an oxygen complex by myoglobin and haemoglobin is itself a significant factor in protecting the haem from oxidation.

In view of these conclusions, reaction 5 is a more likely first stage of the oxidation than reaction 6 which was employed in the detailed mechanism discussed by George & Stratmann (1952*b*); but no distinction can be made between them on purely kinetic grounds. Consideration of the energetics places myoglobin in the same class as the ferrous ion regarding stability towards oxidation, for the ionization potential of ferrous ion in solution is 95 kcal./g.mol., compared with the provisional value of 96 kcal./g.mol. for myoglobin. Yet the oxidation of myoglobin is undoubtedly a faster reaction. Among many possible explanations, a reaction between MbO_2 and a reducing group present in the system could be considered as initiating the oxidation instead of reactions 5 or 6. Such an initiating reaction could easily be more rapid; but no scheme incorporating a reaction of this type has yet been found which would yield the observed kinetic behaviour. Nevertheless, the consumption of oxygen in excess of that required to oxidize the haem iron, described by George & Stratmann (1952*a*), does show that at some stage in the oxidation other groups in the molecule are attacked. This fact, together with the apparent stability of myoglobin according to the energetics, would provide a useful starting point for a future investigation.

SUMMARY

1. The oxidation of myoglobin (Mb) to metmyoglobin (MetMb) by oxygen is inhibited by carbon monoxide. The rate of oxidation in O_2–CO mixtures at atmospheric pressure, with partial pressure of carbon monoxide between 0 and 45 mm., pH 5·7 and 30°, is first order with respect to unoxidized myoglobin. The partial pressure of CO required to halve the uninhibited rate constant was 21 ± 2 mm.

2. The partition constant of myoglobin between CO and O_2 enters into the rate equations for the inhibited oxidation. Provided that a value of about 15 is appropriate to the present experimental conditions the results favour a rate equation containing $(1-\alpha)^2$ for the oxidation at high oxygen pressures, where $(1-\alpha)$ is the fraction of MbO_2.

3. In the pH range 5·35–6·64 at 30°, the rate of oxidation increases with hydrogen-ion concentration at both low and high oxygen pressures. The effect is small in magnitude, and could be due to the ionization of haem-linked groups on the myoglobin molecule.

4. A discussion of the available thermochemical data shows that electron transfer between Mb and O_2 giving MetMb and O_2^- is probably a relatively slow reaction since it would be endothermic to about 20 kcal./g.mol. The electron transfer dissociation of MbO_2 into MetMb and O_2^- would be much less favoured having an endothermicity of about 36 kcal./g.mol. on account of the large exothermic heat of formation of MbO_2. The formation of an oxygen complex by myoglobin and haemoglobin in an exothermic reaction is thus in itself a significant factor in protecting the haem from oxidation.

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